Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Genetics & Development

The role of chromatin insulators in nuclear architecture and genome function

Kevin Van Bortle and Victor G Corces

Eukaryotic genomes are intricately arranged into highly organized yet dynamic structures that underlie patterns of gene expression and cellular identity. The recent adaptation of novel genomic strategies for assaying nuclear architecture has significantly extended and accelerated our ability to query the nature of genome organization and the players involved. In particular, recent explorations of physical arrangements and chromatin landscapes in higher eukaryotes have demonstrated that chromatin insulators, which mediate functional interactions between regulatory elements, appear to play an important role in these processes. Here we reflect on current findings and our rapidly expanding understanding of insulators and their role in nuclear architecture and genome function.

Address
Department of Biology, Emory University, Atlanta, GA 30322, United States

Corresponding author: Corces, Victor G (vcorces@emory.edu)

Current Opinion in Genetics & Development 2013, **23**:212–218

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Genevieve Almouzni** and **Frederick Alt**

For a complete overview see the Issue and the Editorial

Available online 5th January 2013

0959-437X/$ – see front matter,

http://dx.doi.org/10.1016/j.gde.2012.11.003

Introduction

The identity and developmental potential of any given cell begins within the cell nucleus, where spatiotemporal regulation of genome organization and expression underlie the transformation from a totipotent cell into a complex system of tissues and differentiated cell types. This incredible pathway is largely accomplished through the action of functional non-coding regulatory elements, which include enhancers, silencers, promoters, and insulators. Chromatin insulators were first discovered for their ability to protect genes from position effects in transgene assays, and have since been characterized as multi-protein DNA complexes capable of facilitating long-range inter-chromosomal and intra-chromosomal interactions. More importantly, interactions facilitated by insulator proteins typically underlie functional contacts between regulatory elements, such as enhancers and promoters, or chromatin domain organization conducive to coregulation of active or silent genes. These features, combined with microscopy-based and biochemical studies suggesting insulators target associated loci to specific nuclear subcompartments, suggest that insulators are crucial players in constructing appropriate three-dimensional nuclear architecture. Though much of our current understanding of how insulators function comes from studies of CTCF, a highly conserved zinc finger protein capable of insulator activity in vertebrates, studies in other model systems have provided concordant evidence that insulators function by bridging together distant loci, yet the composition and proteins required for activity varies [1,2]. Nevertheless, pinpointing the exact purpose of insulators in genome biology has proven to be difficult, as insulator proteins appear to be involved in a multitude of diverse, context-dependent biological activities. In this respect, we consider recent developments in our understanding of insulators and their roles in nuclear organization and cell differentiation.

Distribution, correlation, and organization of insulators throughout the genome

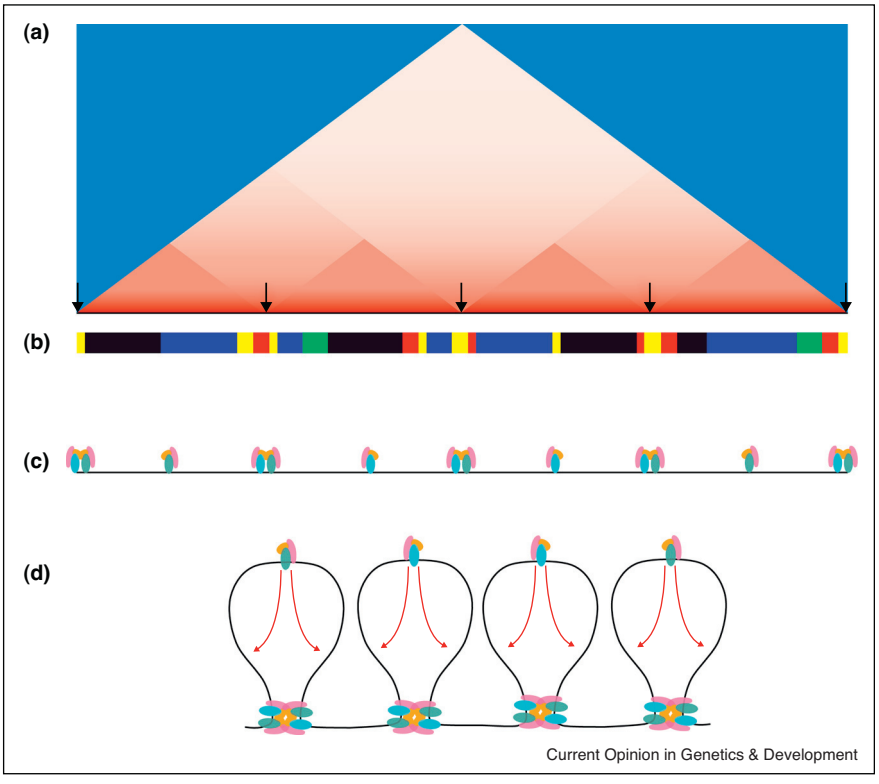
The occupancy landscape of insulator proteins has been mapped genome-wide by combining chromatin immunoprecipitation with microarray hybridization (ChIP-chip) [3–6], high throughput sequencing (ChIP-seq) [7–9], and more recently with even higher precision using ChIP-exo [10]. Insulator proteins localize to thousands of sites characterized by conserved target sequences, wherein differences in DNA motifs can influence protein occupancy levels and features of insulator function [9,11,12]. The CTCF insulator protein localizes to DNase I-hypersensitive sites, characteristic of 'open chromatin', that are generally common across cell types [13]. Detailed comparison of CTCF binding sites across 38 human cell lines suggests that while a majority of insulators are indeed invariant between cell types, thousands of cell-type specific CTCF sites are also present [14•]. Variable CTCF binding sites are associated with differential DNA methylation within the CTCF recognition sequence [15], and whereas ubiquitous CTCF sites predominantly map to intergenic regions, cell-type specific CTCF sites are enriched within the introns of genes [14•]. In addition to CTCF, RNA polymerase III (RNAP III) transcription factor TFIIIC is capable of insulator activity in both yeast and humans [16••], at tRNA genes and RNAP III independent sites where TFIIIC is recruited to highly conserved B-box elements. TFIIIC and CTCF sites associate with the cohesin complex [17–19], which

likely stabilizes long-range interactions and is essential for insulator activity [20,21].

The distribution of insulator proteins initially provided a certain degree of support to previously proposed models, wherein chromatin insulators function as heterochromatin barriers. For example, insulators localize to the borders of some repressive chromatin domains in yeast [20,22], *Drosophila* [23], and mammals [8,14•], suggesting that they might establish a roadblock to prevent the spread of gene silencing, consistent with their ability to protect transgenes from position effects. However, these correlations do not account for a majority of insulator-binding sites and do not explain why insulators only delimit a subset of repressed loci. In light of this discrepancy, recent exploration of the nature and function of insulator proteins in their endogenous contexts point to a role beyond barrier function. For one, depletion of *Drosophila* insulator proteins does not lead to substantial changes in the distribution of H3K27me3, an epigenetic signature of Polycomb (Pc)-mediated repression, at most domain borders [24•]. Meanwhile, mapping of all CTCF-mediated interactions in mouse embryonic stem (ES) cells has offered a complex picture of chromatin domain organization, characterized by distinct underlying epigenetic states and governed by functional long-range insulator–insulator interactions [25••]. Together, these studies would suggest that insulators are involved in establishing the structural arrangement of chromatin domains, but do not actively participate in the delineation of epigenetic status.

Chromosome conformation capture (3C)-based genome-wide interaction studies in *Drosophila*, mice, and human cell lines have also uncovered the structural organization of interphase chromosomes in unprecedented detail [26••,27••,28••], providing new insight into the correlation and possible role of insulators in nuclear architecture. Genomes appear spatially segregated into topological chromatin domains defined by strong interaction frequencies and separated by domain borders characterized by a

Figure 1



Topological domain structure and correlation with epigenetic profiles. **(a)** Cartoon heatmap representation of physical interactions. Interactions frequencies are represented from high (red) to low (blue). Physical domains are identified as having strong interaction frequencies, separated by sites with a dramatic decrease in short-range interactions (arrows — domain boundaries). **(b)** Principle chromatin types defined by the presence of specific chromatin components. Comparison between physical domains (a) with chromatin profiles (b) reveals that physical domains are independent of epigenetic signatures. Sites of active chromatin (yellow, red) correlate with domain boundaries, whereas silent chromatin domains (black, blue, green) are enriched within the interior of physical domains. **(c)** Domain boundaries also correlate with combinations of insulator proteins, whereas thousands of independent insulator sites localize within physical domains. **(d)** Model integrating the identified topological domains with studies examining the nature and function of insulator proteins. Aligned insulator proteins are enriched at chromatin domain boundaries, establishing the framework for chromatin looping. Interior independent insulators presumably mediate short-range interactions within individual physical domains.